SUPPORTING INFORMATION

Magnetic Nanoparticle-based Electrochemical Sensing Platform using Ferrocene-labelled Peptide Nucleic Acid for the Early Diagnosis of Colorectal Cancer

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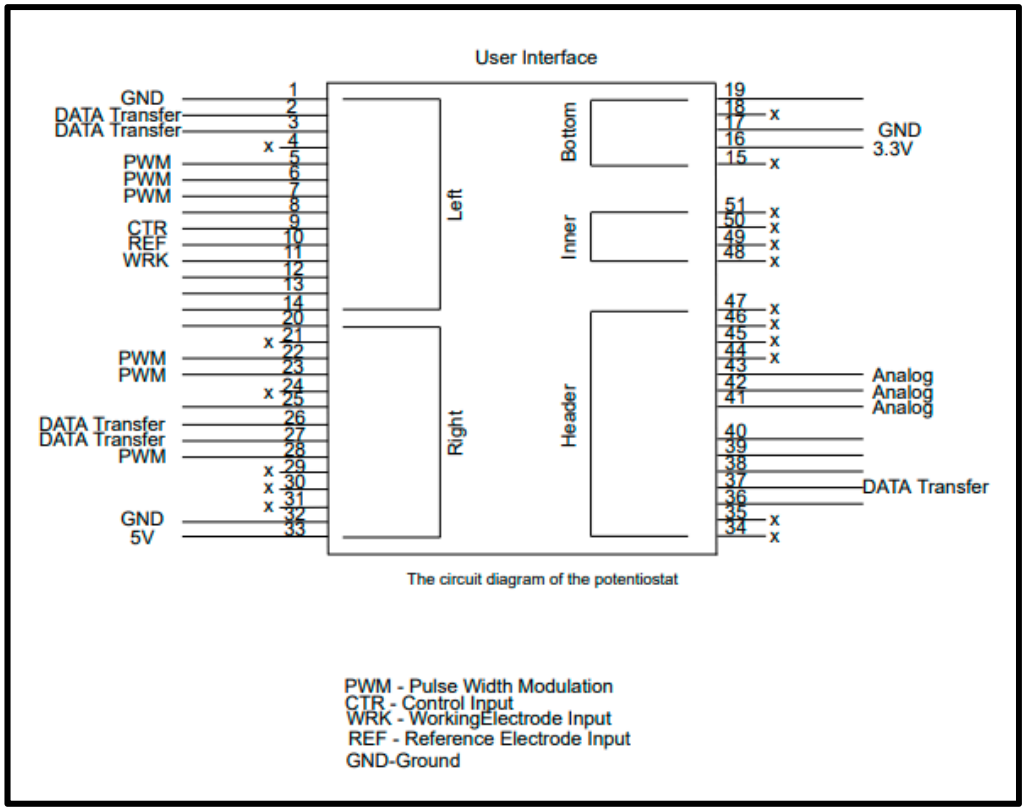


Figure S1. Potentiostat circuit.

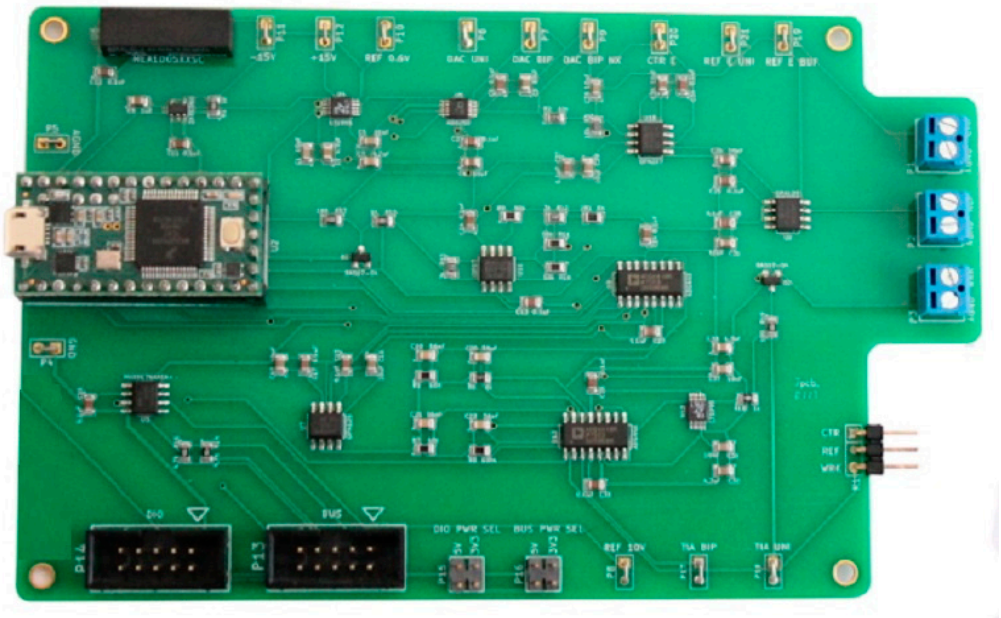


Figure S2. Electrical circuit printing.

Table S1. The sequences used in this study. 5-methylcytosine (5-mC) is indicated with mC.

Name	**Sequence 5′ → 3′**
SEPT9 amplicon 5	AGAAACGCACCATGGTTTGGATGCTGAGGGTGAGGGAAAGAGAGGA GTCAACAGTGGTGCCCGGAGACTTGGCTTGAGCAACTAGGTGGATGG TAGCACCGTTTCCTAAGATGAGGGGCTGTGGGAACTTGAGGGGCTGT GAAGGCTCTGCCACTGACGGACCCAGGGCTCGGCAGCTCTGGGAGGC CTACTTTGTGTCAGGCCACGCAGAGATAAGACCATCTGGGC
target SEPT9	TGGGAACTTGAGGGGCTGTGAAGGCTCTGCCACTGACGGACCCAGGG CTCGGCAGCTCTG
100met_SEPT9	TGGGAACTTGAGGGG**mC**TGTGAAGGCT**mC**TGCCA**mC**TGA**mC**GGACC **mC**AGGGCT**mC**GG**mC**AGCT**mC**TG
75met_SEPT9	TGGGAACTTGAGGGG**mC**TGTGAAGGCT**mC**TGCCA**mC**TGA**mC**GGACC CAGGGCT**mC**GGCAGCT**mC**TG
50met_SEPT9	TGGGAACTTGAGGGGCTGTGAAGGCTCTGCCA**mC**TGA**mC**GGACCCA GGGCT**mC**GGCAGCT**mC**G
25met_SEPT9	TGGGAACTTGAGGGGCTGTGAAGGCTCTGCCACTGACGGACCCAGGG CT**mC**GGCAGCT**mC**TG
BCAT1 promoter	TGCAATCCAGCC**mC**GCCA**mC**GTGTACTCGC**mC**GCCGCCT **mC**GGGCACTGCCCCAGGTCTTGCTG
SOX21-AS1 promoter	GCAGAG**mC**GCAGCAATGGATATAAATACAAATA**mC**AGCAGCAC**mC** AGC**mC**TGTCTTCTTGGCTC

Table S2. ITC measured thermodynamic parameters of the PNA-DNA interaction.

DNA fragment	**K_d (nM)**	**ΔH(kcal/mol)**	**ΔG(kcal/mol)**	**ΔS(kcal/mol)**
unmethylated SEPT9	26.5	-53.2	-10.3	-42.9
100met_SEPT9	85.5	-61.6	-9.65	-51.9
75met_SEPT9	20.8	-51.7	-10.5	-41.2
50met_SEPT9	30.4	-55.3	-10.3	-45.1
25met_SEPT9	9.49	-50.8	-10.9	-39.8

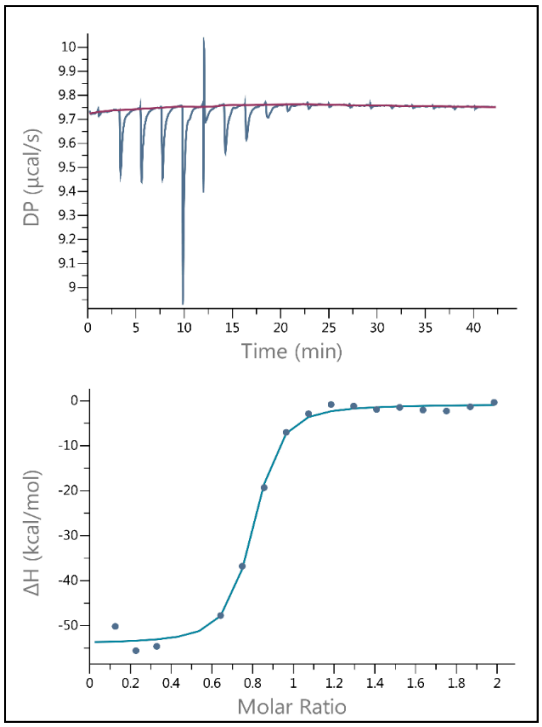


Figure S3. Titration data for the hybridization between unmethylated SEPT9 fragment and PNA. Raw ITC data (top) and enthalpy change (down).

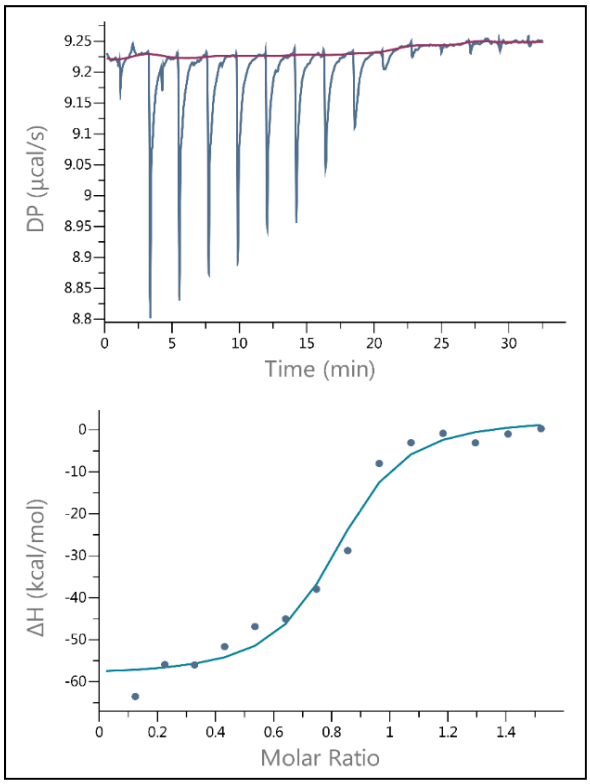


Figure S4. Titration data for the hybridization between 100met_SEPT9 and PNA. Raw ITC data (top) and enthalpy change (down).

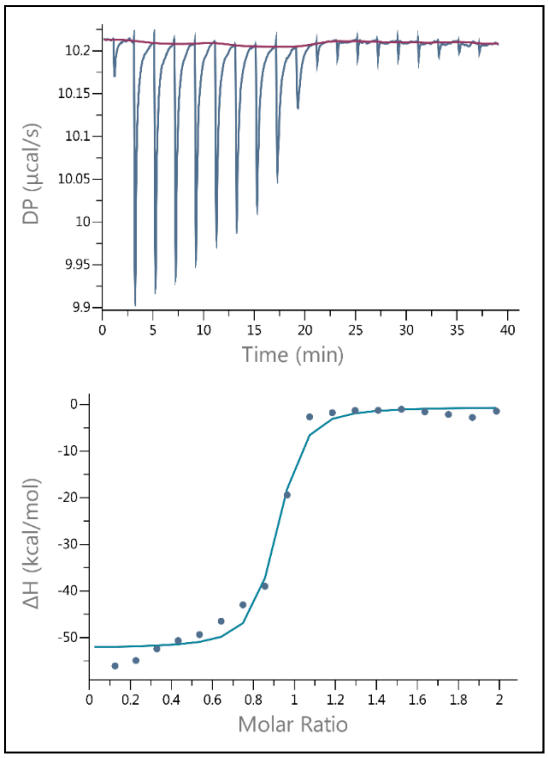


Figure S5. Titration data for the hybridization between 75met_SEPT9 and PNA. Raw ITC data (top) and enthalpy change (down).

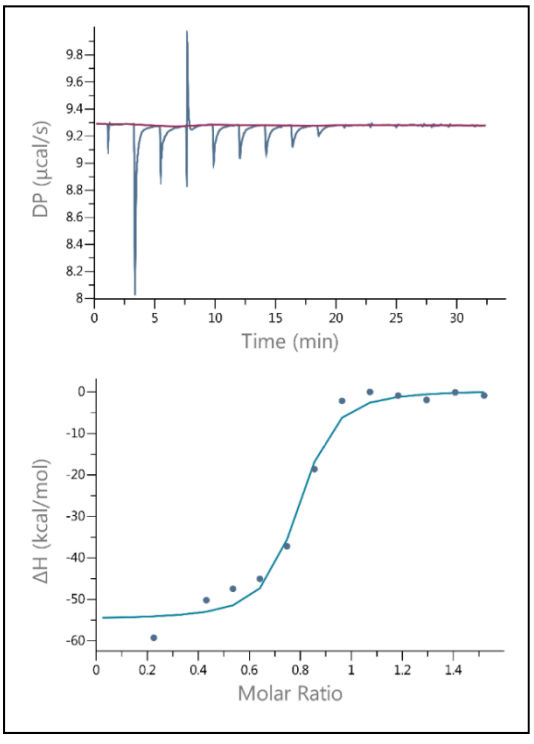


Figure S6. Titration data for the hybridization between 50met_SEPT9 and PNA. Raw ITC data (top) and enthalpy change (down).

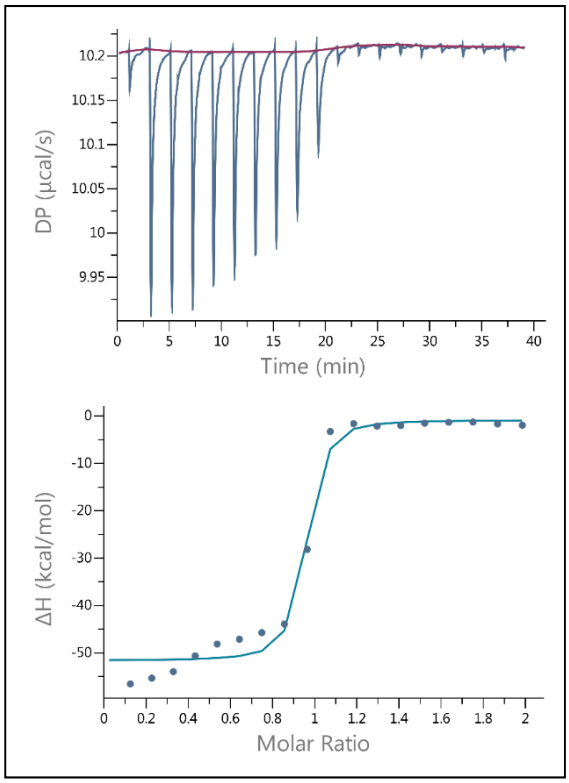


Figure S7. Titration data for the hybridization between 25met_SEPT9 and PNA. Raw ITC data (top) and enthalpy change (down).

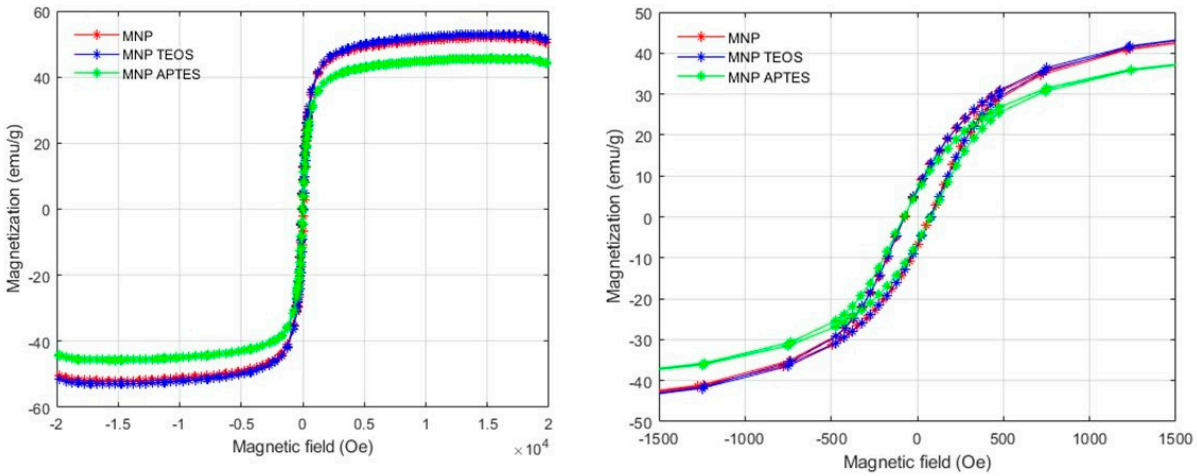
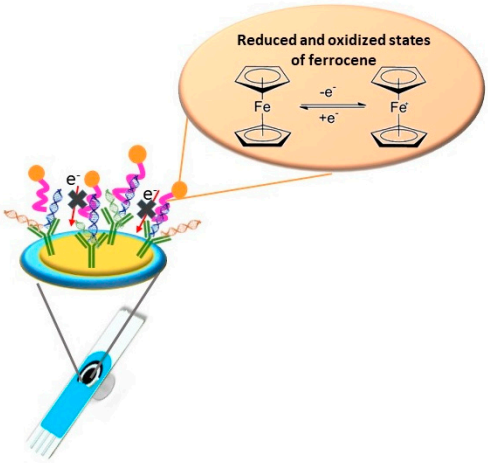


Figure S8. VSM results.

Table S3. Numerical data of CV and EIS measurements of biosensor.

	Cationic Current Values (μA)	Anionic Current Values (μA)	Ohm (Ω)
Bare SPCE	29.288	-38.676	2092
SPCE/MNP	24.009	-33.126	6192
SPCE/MNP/EDC:NHS	22.608	-32.811	5765
SPCE/MNP/EDC:NHS/5-mC Ab	12.318	-22.527	26560
SPCE/MNP/EDC:NHS/5-mC Ab/mSEPT9/Fc-PNA	0.310	-0.589	100000



Scheme S1. Schematic depiction of the detection principle.

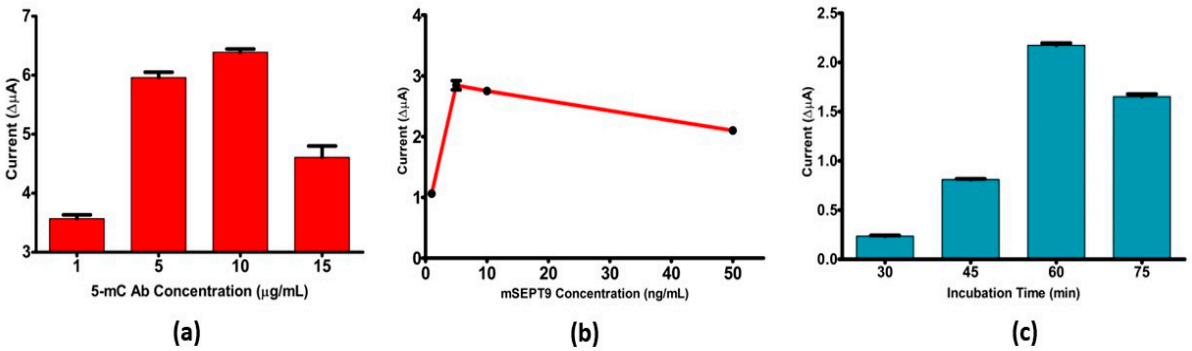


Figure S9. Optimization steps of the proposed CRC diagnosis system. (a) 5-mC Ab concentration, (b) mSEPT9 concentration, and (c) mSEPT9/PNA incubation time.

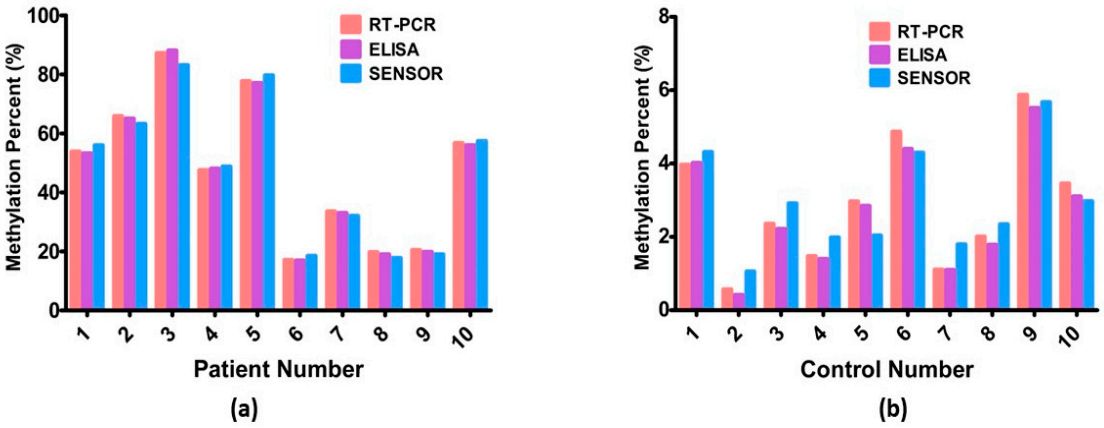


Figure S10. Comparative results for sensor, methylated DNA quantification kit (ELISA) and RT-PCR for (a) Patient samples (n=10), (b) Control samples (n=10).

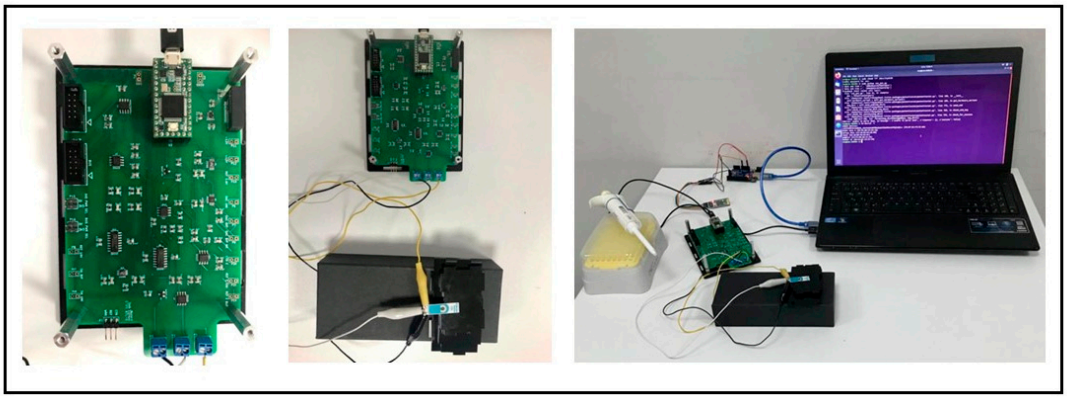


Figure S11. Images of the POC.